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L4: Entry 5 of 16 File: USPT Oct 17, 2000

US-PAT-NO: 6133503
DOCUMENT-IDENTIFIER: US 6133503 A

TITLE: Mammalian artificial chromosomes and methods of using same

DATE-ISSUED: October 17, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Scheffler; Immo E.	Del Mar	CA	N/A	N/A

US-CL-CURRENT: 800/21; 800/24, 800/25

CLAIMS:

What is claimed is:
1. A method of producing a mouse expressing a selectable marker which is present on a mammalian artificial chromosome (MAC), comprising the steps of:
a. introducing a MAC containing the selectable marker into an ovum cell, said ovum cell fertilized into a zygote at the time of or following said introduction of said MAC, wherein said zygote contains said MAC containing said selectable marker;
b. implanting said MAC-containing zygote into a female mammal; and
c. selecting a mouse produced from said zygote, wherein said mouse expresses said selectable marker.
2. A method of producing a mouse expressing a selectable marker which is present on a mammalian artificial chromosome (MAC), comprising the steps of:
a. introducing a MAC containing the selectable marker into an embryonic stem cell;
b. introducing said MAC-containing embryonic stem cell into an embryo;
c. implanting said MAC-containing embryo into a female mammal; and
d. selecting a mouse produced from said embryo, wherein said mouse expresses said selectable marker.
3. The method of claim wherein said MAC is less than about 0.1% of the size of the normal haploid genome of the mammalian cell from which the centromere was obtained.
4. The method of claim 1, wherein said MAC comprises a unique cloning site, which comprises a nucleic acid sequence encoding said selectable marker.
5. The method of claim 1, wherein said selectable marker is an

exogenous nucleic acid sequence.
6. The method of claim 2, wherein said MAC is less than 0.1% of the size of the normal haploid genome of the mammalian cell from which the centromere was obtained.
7. The method of claim 2, wherein said MAC comprises a unique cloning site, which comprises a nucleic acid sequence encoding said selectable marker.
8. The method of claim 2, wherein said selectable marker is an exogenous nucleic acid sequence.
9. A method of producing a transgenic mouse expressing a selectable marker which is present on a mammalian artificial chromosome (MAC), comprising the steps of:
a. introducing a MAC containing the selectable marker into an embryonic stem cell by microcell fusion of an embryonic stem cell and a second cell which contains the MAC to produce a hybrid cell expressing the selectable marker;
b. introducing said MAC-containing hybrid cell into an embryo;
c. implanting said MAC-containing embryo into a female mammal;
d. selecting a mouse produced from said embryo, wherein germ cells of said mouse express said selectable marker;
f. mating said mouse with another mouse to produce progeny; and

g. selecting a transgenic mouse from said progeny.
10. The method of claim 9, further comprising selecting said hybrid cell by culturing hybrid cells under conditions to select for expression of the selectable marker.
11. The method of claim 9, wherein said MAC comprises a unique cloning site.
12. The method of claim 11, wherein said unique cloning site comprises a nucleic acid sequence encoding said selectable marker.
13. The method of claim 9, wherein said MAC is less than 0.1% of the size of the normal haploid genome of the mammalian cell from which the centromere was obtained.
14. The method of claim 9, wherein said selectable marker is an exogenous nucleic acid sequence.